Fall '14 **BCH370** Name KEY
Hackert **HW- 2 (20 pts)** UTeID _____

1. Consider a FRET experiment where the measured efficiency of energy transfer between two chromophores is 45%. If R_o = 35.0 Å, **estimate the separation** of the two chromophores. (R = 36.2 Å)

(2) $0.45 = \frac{1}{(1+x^6)}$ where $x = r/r_0$; $x^6 = \frac{0.55}{0.45} \Rightarrow x = \sqrt[6]{1.22} = 1.03$; $R = 1.03\,R_0$

2. Balance the following radioactive decay equation by filling in the blank with the missing item.

(1) a) $^{206}_{81}Tl \rightarrow {}^{206}_{80}Hg + {}^{0}_{+1}\beta^{+}$

(1) b) A radioisotope I-131 has a half-life of 8.06 days. Calculate the decay rate constant of the radioisotope.
Rate constant (with units) = 0.0860 d^{-1} $k = \frac{\ln 2}{t_{1/2}} = \frac{0.693}{8.06\,d} = 0.0860\,d^{-1}$

(1) c) The how many **days** will it take for 98% of a sample of I-131 radioisotope rated at 30 microCuries to undergo radioactive decay ? 45.5 days **days.** $A = A_0 e^{-kt} \Rightarrow 2 = 100\,e^{-kt}$; $\ln 50 = kt$

3. SDS gels are greatly improved in resolution by running a "stacking" gel and a "resolving" or "running" gel.
a) **Name two key property differences** between the "stacking" gel and the "resolving" gel that contribute to the improved resolution of running DISC PAGE.

(1) a) pH: stacking gel is lower pH (~6.9) where Gly ~0 charge)

b) %gel: stacking gel is lower % acrylamide – separate in running gel

What is the role of each of the following in performing SDS-PAGE?

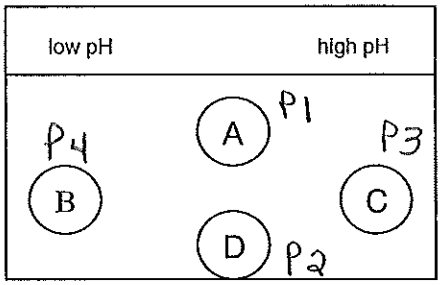
(1)
a) Bromophenol Blue – tracking dye to follow front of running gel

b) Coomassie Blue: – protein stain added after run to visualize proteins

4. You performed a 2D IEF-SDS PAGE experiment on peptides P1, P2, P3, and P4 and obtained the following results. Match each spot with its peptide number.

A = P1; B = P4; C = P3; D = P2.

(2)
P1= FAGRRALVEDPIW largest
P2= RACKED smallest
P3= IKLRGAKPV high pK_a
P4= AGWDPLEFD low pK_a



5. The equation of motion for a small, spherical particle of mass (m) and frictional coefficient (f) that is initially at rest, and then acted on by a constant force (F) at time t = 0 is F –fv = ma. (From calculus recall that F - fv = m(dv/dt) solves to v = (F/f) [1 - exp(-ft/m)].)
a) Show that such a particle will initially accelerate but over time will approach a "maximal" velocity.

(1) $v = \frac{F}{f}(1 - e^{-ft/m}) \rightarrow$ as $t \rightarrow \infty$, $e^{-ft/m} \rightarrow 0 \Rightarrow v \rightarrow F/f$ (constant)

(1) b) Consider protein molecule that is assumed to be spherical with a diameter of 66Å, a density of 1.32 g/cm^3 and a v-bar of 0.72 cm^3/g. Calculate the expected diffusion constant for this protein (Assume T = 20° C and η = 0.01 (g/cm-s). $6.5 \cdot 10^{-7}$ cm^2/s $D = RT/N \cdot f$ (calc. f to get D)

a) $f_{sph} = 6\pi\eta \cdot R_{sph}$

$f_{sph} \sim 6\pi(0.01\ \frac{g}{cm \cdot s})(33 \cdot 10^{-8}\ cm) = 6.2 \cdot 10^{-8}\ g/s$

$D = \frac{8.314 \cdot 10^7\ \frac{g \cdot cm^2}{mol \cdot s \cdot K}\ (293\ K)}{6.02 \cdot 10^{23}/mol\ (6.2 \cdot 10^{-8}\ g/s)}$

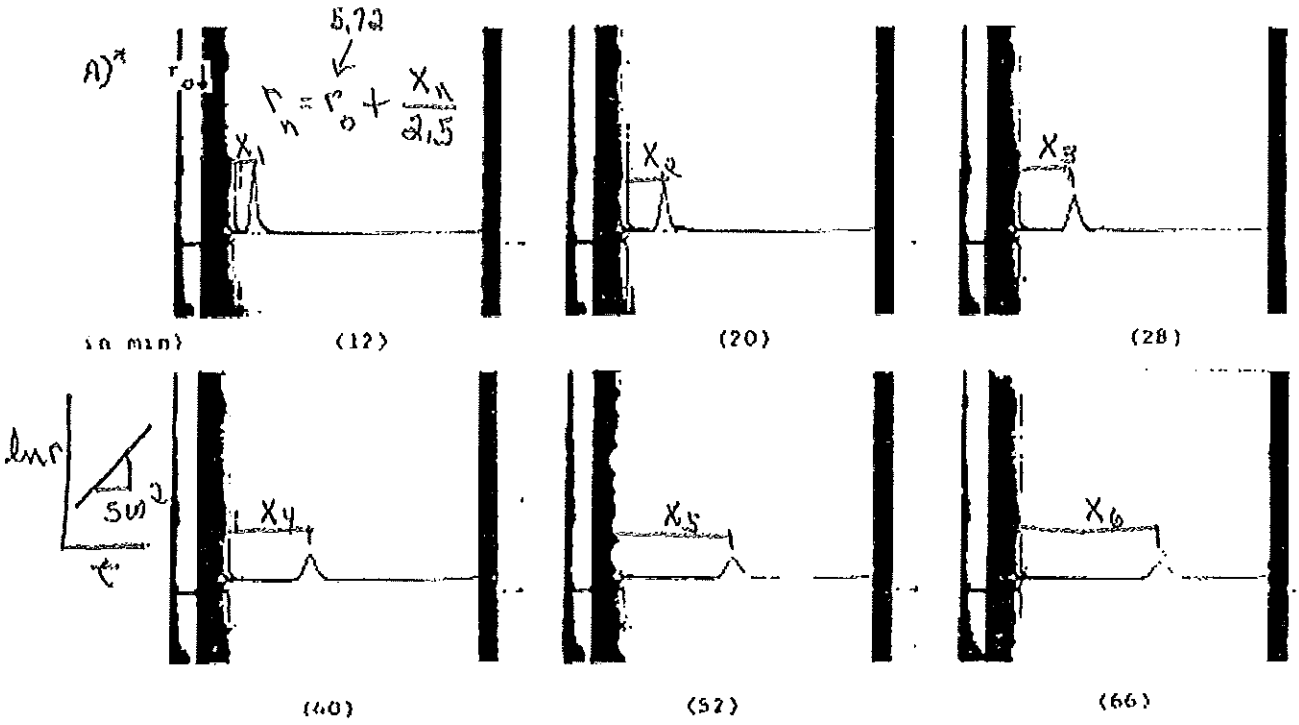
6. What is typically measured by dynamic light scattering (LS)? D → f → R_h (hydrodynamic radius)
(1)
What wavelengths are normally employed in making circular dichroism (CD) spectra? 180 - 240 nm

7. Determine the sedimentation coefficient (s) and molecular weight (M) for the sample that gave the following data when subjected to: A) a sedimentation velocity run using Schlieren optics, and B) a sedimentation equilibrium run using interference optics.

Note: the figures below have been magnified to allow you to make measurements from the figures. The "r" can be determined from the reference points (r_o) and the magnification factors. Assume T = 20° C, density of buffer = 0.9968 g/mL, and v-bar = 0.717 cm^3/g for the protein, and η = 0.01 (g/cm-s) for both experiments.

A) Sed. Vel. : ω = 36,000 rpm, magnification factor (2.5X), r_o = 6.72 cm. (times are given in minutes).

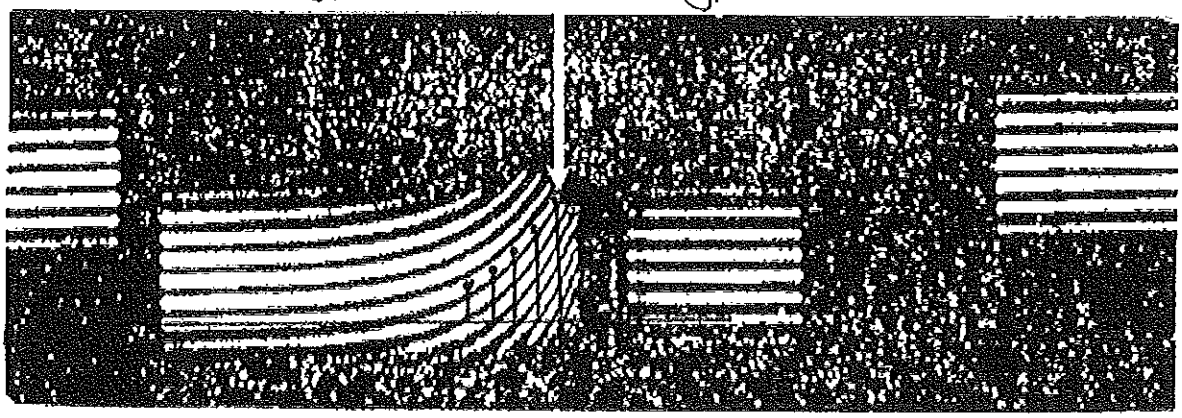
(4) Report "s" in proper units [s = $\approx 23 \cdot 10^{-13}$ s ≈ 23 S] (Show work and attach plot).



B) Sed Equilibrium: ω = 5000 rpm, magnification factor (25X), r_o = 6.75 cm. Calculate M in g/mol (4pts) and

(4) also estimate the concentration of the protein at the position with the white arrow (1 pt). Assume the cell path length to be 12.00 mm, λ = 546 nm, and (dn/dc = 0.186 $(g/cm^3)^{-1}$.

[M = $\approx 1.0 \cdot 10^6$ g/m ; [ ]$_{arrow}$ = ≈ 1.4 mg/mL] (Show work and attach plot).



I hereby declare that I did this assignment independently: _____